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EFFECT OF SOIL RE-OXIDATION ON WHEAT (*TRITICUM AESTIVUM* L.) DEFENSE SYSTEM*

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A b s t r a c t. Responses of superoxide dismutase (SOD) activity, malonic di-aldehyde (MDA) level and pigments (chlorophyll, carotenoids) content were evaluated in roots and leaf tissues of wheat plants (Roma; CZR 1334) which were grown in soil (Eutric Cambisol) pre-exposed to anoxia by flooding, during return of oxic conditions in the root zone.

K e y w o r d s: soil aeration, oxygen stress, *Triticum aestivum* L., superoxide dismutase, malonic dialdehyde, pigments, redox potential, oxygen diffusion rate

INTRODUCTION

Soil aeration status is an effect of equilibrium between biological processes of oxygen uptake and carbon dioxide production in the soil environment and physical processes of gas transport between the soil and the atmosphere. The main components of the soil air are oxygen and carbon dioxide [7].

The physical process of gas transport depends directly on its diffusion coefficient in the soil. In the well-drained soils, pore spaces are filled with air and are interconnected with the atmosphere above the soil. Thus the thin moisture films around individual soil particles contain appreciable amount of dissolved oxygen as a result of the presence of oxygen in the gas-filled pore space. The transport rate of oxygen in a well-drained soil is sufficient to supply all of the oxygen required for microbial and plant root respiration as well as chemical oxidative reactions [6].

When the soil is flooded for long intervals of time and the pore space is completely filled with water, oxygen consumption by chemical and biological processes continues as long as some oxygen is available, but gaseous diffusion of oxygen into the profile is not possible. Within a period of few hours to a few days, depending mostly on the energy source available for microorganisms, soil oxygen levels can be depleted to such an extent that alternative electron acceptors must be used for microbial respiration. At this point the soil is said to become anaerobic.

The contribution of the indirect effects of soil oxygen depletion, i.e., changes in the soil nutrient availability, in soil pH, toxic metabolites, and in the activity of soil microflora and microfauna, to the total plant response to anoxia depends predominantly on the soil redox potential changes and on the soil redox buffering capacity [13,14].

Various degrees of anaerobiosis, including anoxia, i.e., the complete absence of molecular oxygen, occur in the soil. Soil oxygen

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plays a fundamental role in the physiology of the roots and plants vary widely in their response to O_2 stress in the soil, as it is known that there is a wide spectrum of plants from hygrophytes through mesophytes to xerophytes.

Most of the cultivated plants, except rice, are mesophytes, but differentiation among them is significant [1].

For example, flooding of wheat for 30 days during grain filling at the soil temperature of 15 and 25°C reduced grain yield by about 20 and 70%, respectively [10].

The changes in specific environmental factors are known to induce or activate enzymes in higher plants [4,15], and it is expected that the levels of enzymes associated with the protective system, that prevents oxidative injury, would be regulated to accomodate the soil oxygen availability for plant roots.

Cells contain antioxidants, such as carotenoids, which interrupt free radical chain reactions and thus decrease the damage done per radical initiator [5].

The superoxide dismutase (SOD), a family of metalloenzymes (EC 1. 15.1.1) known to accelerate spontaneus transformation of free superoxide radicals, play a central role in the defence mechanism of the organism against the reactive oxygen species that have been found to be produced during the environmental adversity [2].

Many plants are able to survive a period of anoxia only to die upon subsequent re-exposure to air, suggesting that oxidative damage may occur during the recovery phase [8]. An increased SOD activity is suggested to be vital in protecting plants against oxidative stress generated upon re-exposure to air.

The aim of the experiment was to investigate to what extent the SOD activity and the presence of pigments disactivate free superoxide radicals and affect the level of malondialdehyde (MDA) - a product of oxidative destruction of membrane lipids, during return of oxic conditions in the root zone of plants pre-exposed to soil anoxia.

MATERIAL AND METHODS

The soil aeration conditions in the root zone were characterized with such indicators as oxygen diffusion rate (ODR) and redox potential (Eh).

The experiment was performed with the use of two winter wheat cultivars (Roma, CZR 1334).

The plants were subjected to oxygen stress for 20 days by flooding the soil. After 20 days the excess of water was removed and the measurements of soil aeration status, SOD activity, MDA level and pigments content were performed, and repeated after 2 and 8 days. These values in relation to the soil oxygenation indicators are presented.

Soil material

The experiment was performed in plastic pots, 5.9 dm^3 in volume, filled with soil. The soil was collected from an agricultural area at Elizówka near Lublin, from Ap horizon of brown loess soil (Eutric Cambisol). The soil characteristics are shown in Table 1.

Table 1. Eutric Cambisol characteristics

pH (H_2O)	7.3
pH (KCl)	7.1
organic carbon	0.89 %
1-0.1 mm fraction	15.00%
0.1-0.002 mm fraction	80.00%
clay	5.00%

Each pot contained 6.2 kg of soil (dry basis) packed to the bulk density 1.35 Mg m^{-3} corresponding to total porosity of 48% v/v. The soil volume in the pot was 5.0 dm^3.

Plant material

Wheat seeds (cultivars Roma and CZR 1334), were sown 20 in each pot. After germination the seedlings were placed in a growth chamber with a photon flux density of about 775 $\mu m^{-2}s^{-1}$ at the leaf level, and photoperiod of 16/8 h.

The air temperature was 25±1°C, and during the night it was 12±1°C, the relative humidity in the growth chamber was 45±5% during the day and 70±5% during the night. After the first 8 days the seedlings were thinned to 5 per pot.

During the first 18 days of plant growth, the water content was kept within the limit of 19-22% by weight, corresponding to the soil moisture tension of 50-20 kPa, i.e., in the range of water easily available for plants. This gave an air-filled porosity of 25±2%. After 18 days of plant growth at the phenological stage of 3 leaves, the oxygen conditions in the soil were differentiated by increasing soil moisture content with destillated water, up to the soil saturation level. Thus, two treatments were obtained with the following air-filled porosities: Eg 25±2% (control), and Eg 0% (full saturation with 5-10 mm of stagnant water on the soil surface). The stress conditions were maintened for 20 days.

Soil measurements

After 20 days from the stress onset the excess of water was removed from the Eg 0% treatment, and the re-oxidation of the soil started on.

In order to characterize the soil aeration conditions, the measurements of ODR characterizing the oxygen availability for plants, and of Eh were performed at the moment of the soil oxygen stress interruption and after 2 and 8 days.

The ODR values were measured by a device with an automatic adjustment of the effective reduction voltage, described by Malicki and Walczak [11], using five 0.5x4 mm platinum electrodes placed in the soil and polarized to the voltage of -0.65 V versus saturated calomel electrode during four minutes.

Redox potential was measured with five similar electrodes, placed at the same depth, with a portable Orion 404 ion-analyzer.

Plant measurements

The plant response to re-exposure to good soil aeration conditions was characterized by the measurements of SOD activity and of MDA level within the roots and leaves, and of the pigment content in leaves. The measurements were performed: at the onset of soil drying period (control values) and repeated after 2 and 8 days.

After ODR and Eh measurements, the plants of one of the flooded treatments were removed, their roots washed out and weighed.

For the SOD determination, a homogenate of the plant roots and leaves was used. The leaf homogenate was prepared in three replications (of three differents plants) using the leaf middle part. The root homogenate was prepared in three replications of the averaged root sample (each of a separate plant). A half of the sample was homogenized in a mortar cooled in a refrigerator to 2-4 °C with 4.5 cm^3 of 5 mM Tris-HCl buffer (pH 7.4). The homogenate was filtered through capronic tissue and then used for determinations. For the SOD determination in the leaves, the homogenate was diluted 20 fold, and in case of the roots, 5 fold, by adding the buffer solution. The determination was performed according to Paoletti *et al.* [12] using a spectrophotometric method based on the inhibition of oxidation of NADH by superoxide radicals. A decrease of the NADH oxidation rate is a function of the enzyme activity. The measurements were performed in quartz cuvettes 3 ml in volume with the UV-Vis spectrophotometer (Shimadzu UV-Vis 160A) at the wave length 340 nm. As a unit of SOD activity 50% inhibition of the NADH oxidation rate was used.

The level of MDA was determined using the original homogenates of the roots and leaves (without dilution) using the test with 2-tiobarbituric acid according to Dhindsa and Matowe [3] but with the introduction of butanol extraction step for the removal of interfering compounds.

The content of chlorophylls and carotenoids in the leaves was determined by the spectrophotometric method of Lichtenthaler and Wellburn [9] using pure acetone extracts with three biological replications.

RESULTS AND DISCUSSION

After 20 days of waterlogging, at a soil mean temperature of 18.5 °C±1 °C, Eh reached the values of 137-161 mV, and ODR 1.5-2.0 $\mu g\ m^{-2}\ s^{-1}$. This indicates, that complete soil anoxia status was reached when the flooding ceased.

The dynamic of the wheat SOD, MDA, and pigments during the restoration of good aeration status in the soil with respect to leaves and to roots is shown in the Figs 1-3.

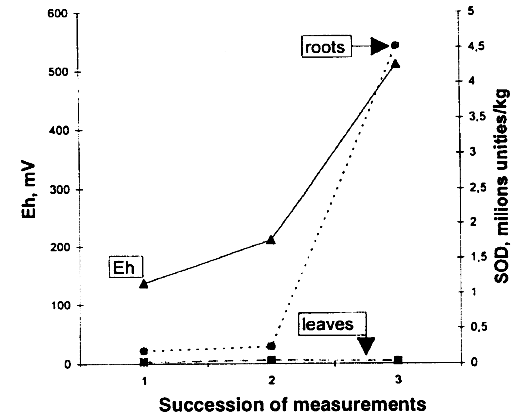

Fig. 1. The SOD activity in wheat plants during soil reoxidation (cv. CZR 1334).

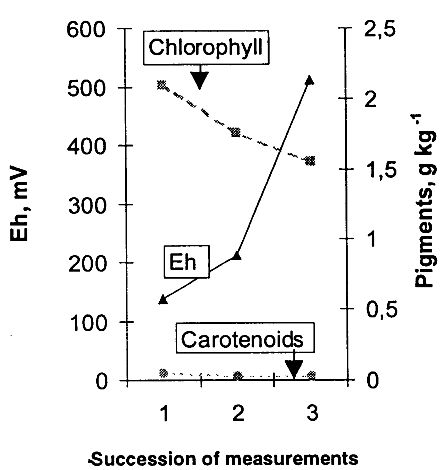

Fig. 2. The pigment content in the leaves of wheat during soil reoxidation (cv. CZR 1334).

When the flooding ceased, the supply of O_2 to the soil by gas diffusion re-started. All the values of soil aeration indicators increased and after 8 days they established at the control values.

The response of the stressed plants to the restoration of oxic conditions in the root zone of the soil was evaluated by regression of the physiological parameters such as SOD activity, MDA content and pigment level versus the soil aeration indicators, Eh and ODR.

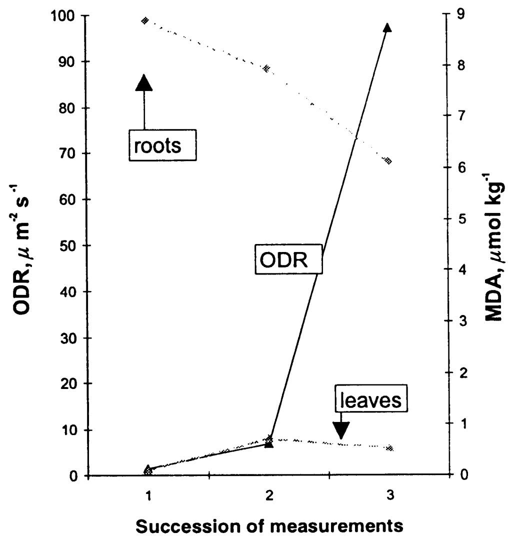

Fig. 3. The MDA level in wheat plants (cv. CZR 1334) during soil reoxidation.

The correlation coefficients for plant SOD and MDA in roots and leaves, and for pigment content in leaves, versus the indicators of soil aeration status were calculated using three models: L - linear: $y = a+bx$, M - multiplicative: $y = ax^b$, and E - exponential: $y = \exp(a+bx)$. The Table 2 provides informations about these data.

They were usually negative with respect to all the indicators of the soil aeration status. Only SOD in leaves for the cv. Roma and in roots for the cultivars Roma and CZR 1334, were positively correlated with the aeration status.

The correlations of the parameters studied with redox potentials, reflecting oxidation-reduction status of the soil medium, were significant ($-0.68<r<0.97$) with the exception of the SOD in leaves.

The correlations with ODR, being an index of direct availability of oxygen for plant roots, turned out to be significant ($-0.70<r<0.99$) with the exception of the SOD in leaves. The highest correlation coefficient with ODR was observed in the case of SOD in roots, as well as with Eh (cv CZR 1334); in both cases they were linear and positive.

Table 2. Correlation coefficients of the plant (*Triticum aestivum* L.) parameters studied with the soil aeration indicators

Plant parameter (g dry mass)	Soil oxygen indicator	Leaves CZR 1334	Roots CZR 1334	Leaves Roma	Roots Roma
SOD	ODR	n.s.	0.99*** L	0.94** E	n.s.
	Eh	n.s.	0.97*** L	0.93** E	n.s.
MDA	ODR	-0.74* E	-0.92** L	-0.80* M	-0.73* E
	Eh	-0.75* E	-0.91** M	-0.77* M	-0.72* E
Chlorophyll	ODR	-0.70* M	-	-	-0.90** M
	Eh	-0.68* M	-	-	-0.86** M
Carotenoids	ODR	-0.80* M	-	-	-0.87** M
	Eh	-0.79* M	-	-	-0.84** M

n.s. - not significant, * - significant at p=0.05, ** - significant at p=0.01, *** - significant at p=0.001, E - exponential, L - linear, M - multiplicative.

MDA in roots was correlated negatively with both the indicators of soil aeration status. The highest correlations with ODR (-0.92) and with Eh (-0.91) were obtained for the multiplicative model. The MDA level in leaves was also negatively correlated both with ODR (-0.74) as well as with Eh (-0.75) for the exponential model.

The pigment content in leaves was correlated negatively both for CZR 1334 and Roma cultivars (multiplicative model), the highest value (-0.90) was observed for chlorophyll with ODR (cv. Roma).

CONCLUSIONS

The experiment performed permitted to indicate that:

1. There exists a close relationship between SOD activity, pigment content and MDA levels in wheat plants, and the changes in the oxygen conditions of an Eutric Cambisol subjected to complete flooding and drying under the conditions of a model experiment.

2. The SOD activity in leaves showed a slight increase after two days from the end of flooding the soil, when the Eh and ODR values reached respectively: 200 mV and 6 $\mu g\ m^{-2}\ s^{-1}$, without further significant alterations; the SOD activity in roots showing a continous increase during 8 days up to 250% of the initial value.

3. Out of the pigments studied only chlorophyll content showed a decrease after 2 days from the drying onset and later stabilized.

4. The content of MDA in roots showed a stepwise decrease after the end of the flooding period without a significant alteration in the leaves.

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